The Laryngoscope
© 2015 The American Laryngological,
Rhinological and Otological Society, Inc.

Systematic Review

Detection of Human Papillomavirus in Laryngeal Squamous Cell Carcinoma: Systematic Review and Meta-analysis

Ricardo Ribeiro Gama, MD, PhD; André Lopes Carvalho, MD, PhD; Adhemar Longatto Filho, PhD; Anderson Paulo Scorsato, BSBIO, MA; Rossana V. Mendoza López, PhD; Jaana Rautava, DDS, PhD; Stina Syrjänen, DDS, PhD, FDSRCSEd(hon); Kari Syrjänen, MD, PhD, FIAC

Background: Recent studies have reported a human papillomavirus (HPV) prevalence of 20% to 30% in laryngeal squamous cell carcinoma (LSCC), although clinical data on HPV involvement remain largely inconsistent, ascribed by some to differences in HPV detection methods or in geographic origin of the studies.

Objective: To perform a systematic review and formal meta-analysis of the literature reporting on HPV detection in LSCC.

Methods: Literature was searched from January 1964 until March 2015. The effect size was calculated as event rates (95% confidence interval [CI]), with homogeneity testing using Cochran's Q and I^2 statistics. Meta-regression was used to test the impact of study-level covariates (HPV detection method, geographic origin) on effect size. Potential publication bias was estimated using funnel plot symmetry.

Results: One hundred seventy nine studies were eligible, comprising a sample size of 7,347 LSCCs from different geographic regions. Altogether, 1,830 (25%) cases tested HPV-positive considering all methods, with effect size of 0.269 (95% CI: 0.242 to 0.297; random-effects model). In meta-analysis stratified by the 1) HPV detection technique and 2) geographic study origin, the between-study heterogeneity was significant only for geographic origin (P = .0001). In meta-regression, the HPV detection method (P = .876) or geographic origin (P = .234) were not significant study-level covariates. Some evidence for publication bias was found only for studies from North America and those using non–polymerase chain reaction methods, with a marginal effect on adjusted point estimates for both.

Conclusions: Variability in HPV detection rates in LSCC is explained by geographic origin of study but not by HPV detection method. However, they were not significant study-level covariates in formal meta-regression.

Key Words: Laryngeal cancer, papillomavirus, meta-analysis, meta-regression, study heterogeneity, publication bias, detection method, geographic region.

Level of Evidence: NA

***Laryngoscope*, 126:885–893, 2016**

INTRODUCTION

The causal relationship between high-risk human papillomavirus (HPV) infection and head and neck squamous cell carcinoma (HNSCC) is well established, following the reports of Syrjänen and coworkers, who first suggested this association more than 3 decades ago.[1] Since then, several epidemiologic and biological studies have implicated HPV infection, mainly the HPV 16 genotype, as a potential etiological factor for the development of tumors in the upper aerodigestive tract, especially in the oropharynx.[2–6] Conversely, the role of high-risk HPV infection in nonoropharyngeal HNSCC is still under discussion, despite an ever-increasing volume of studies and meta-analyses published during the past decades.

The majority of HPV-related squamous cell carcinomas (SCC) arise in the oropharynx (i.e., tonsils and base of the tongue), where the reticulated lymphoepithelium is susceptible to HPV infection–associated oncogenesis.[7] Current literature reports an HPV prevalence greater than 50% in tonsillar carcinomas,[2] and recent studies,

Additional Supporting Information may be found in the online version of this article.

From the Department of Surgical Oncology, Head and Neck Surgery (R.R.G., A.L.C.), Barretos Cancer Hospital, Barretos, Brazil; Laboratory of Medical Investigation (LIM) 14, Department of Pathology (A.L.F.), University of São Paulo School of Medicine, São Paulo, Brazil; Life and Health Sciences Research Institute (ICVS) (A.L.F.), School of Health Sciences, University of Minho, Braga, Portugal; ICVS/3B's–PT Government Associate Laboratory (A.L.F.), Braga/Guimarães, Portugal; Molecular Oncology Research Center (A.L.F.); Department of Bioestatistics (A.P.S.), Barretos Cancer Hospital, Barretos, Brazil; Oncology Translational Centre (R.V.M.L.), Cancer Institute of São Paulo, São Paulo, Brazil; Department of Oral Pathology and Radiology (J.R., S.S.), Institute of Dentistry, University of Turku, Turku, Finland; Department of Clinical Research (K.S.), Biohit HealthCare Plc, Helsinki, Finland.

Editor's Note: This Manuscript was accepted for publication September 22, 2015.

The authors have no funding, financial relationships, or conflicts of interest to disclose.

Send correspondence to Ricardo Ribeiro Gama, PhD, Department of Surgical Oncology, Head and Neck Surgery, Barretos Cancer Hospital, Barretos, Brazil. E-mail: ricardorgama@yahoo.com.br

DOI: 10.1002/lary.25738

particularly those in the United States, show that up to 80% of oropharyngeal SCCs are HPV related.[8] However, only some 40% of these tumors are transcriptionally active with HPV 16 E6 and E7 expression.[8] Conversely, even though high-risk HPV can be found in a minority of SCCs from nonoropharyngeal sites (e.g., oral cavity, hypopharynx, nasopharynx, and larynx), using a rigorous definition, the truly transcriptionally active HPV rates are generally low for oral cavity and larynx cancer—16.3% and 8.6%, respectively.[9]

Laryngeal squamous cell carcinoma (LSCC) represents the second most common malignancy of the head and neck worldwide,[10,11] with 151,000 incidence cases diagnosed worldwide in 2008.[12] Traditionally, LSCC is ascribed to tobacco and alcohol exposure. However, molecular evidence has linked HPV, particularly HPV-16, in the pathogenesis of LSCC.[6] Recent meta-analyses have reported HPV prevalence of 20% to 30% for LSCC.[5,13–15] However, the mere presence of HPV DNA in a tumor does not necessarily indicate that the virus is driving or contributing to tumor development or progression.[16] Methods detecting transcriptionally active HPV, including CDKN2A (p16) immunohistochemistry, quantitative polymerase chain reaction (PCR) or in situ hybridization for HPV DNA or E6/E7 mRNA, might be necessary to demonstrate a biologically active virus.[16–18]

The HPV detection rate among LSCC samples has remained highly variable, ranging from 0% to 85%.[14,19] Some authors have reported that HPV prevalence in LSCC can vary geographically and by ethnic group.[3,6,14,20–22] possibly due to genetic diversity or environmental and cultural differences.[20] Besides geographic and ethnic differences, the disparity in HPV prevalence usually seen among studies is due to an inadequate distinction of patients with LSCC from those with other cancers of the head and neck region, such as oropharyngeal SCC.[4] Additionally, differences in sensitivity and specificity of HPV genotyping methods and diagnostic criteria,[3,23] in addition to the limited spectrum of HPV types analyzed, as well as differences among HPV, have contributed to the inconsistent results regarding the HPV prevalence rate in LSCC.

Herein, a systematic review and formal meta-analysis was performed on HPV prevalence in LSCC, covering all reports published until March 2015, without any restrictions concerning the HPV detection method and geographic origin of the study. The main objective was to estimate the overall HPV prevalence in LSCC and to identify whether inconsistent results among studies are due to different geographic locations and/or HPV detection methods.

MATERIALS AND METHODS

Data Abstraction

We identified eligible studies by searching MEDLINE and reference lists from original articles, book chapters, and reviews from January 1964 until March 2015. No language limitations were imposed. The search terms included: papillomavirus, HPV, larynx, squamous cell, verrucous carcinoma, basaloid squamous cell, papillary squamous cell, cancer, carcinoma, head and neck cancer. All publications that appeared in peer-reviewed journals were eligible, irrespective of HPV detection method. Furthermore, the identified reports had to include exact numbers of analyzed cases and those testing HPV-positive.

Altogether, 783 abstracts were derived from the database, covering the years 1964 through 2015. This abstract list was cross-checked against the other databases (Embase and Cochrane) to find out whether any potential studies were missed in MEDLINE. No such case was identified; however, many of the studies detected in MEDLINE were missing in these two databases. Thus, the final selection was based on these 783 abstracts.

For the present meta-analysis, a total of 179 original studies were determined eligible, fulfilling the above-defined criteria. The vast majority of the ineligible studies surveyed were case reports, case series, or clinical and/or epidemiological studies (including treatment reports), without HPV testing, or were studies on HPV in laryngeal papillomas. The formal meta-analysis was focused on LSCC only, where the different histological types: 1) SSC, 2) basaloid squamous cell carcinoma, 3) verrucous carcinoma, 4) spindle squamous cell carcinoma, and 5) papillary squamous cell carcinoma were treated as subgroups within the study. In final analyses, all subtypes were combined, because all represent LSCC variants.

From the summaries and/or body texts of each eligible study, we extracted the following data: histological types of cancer, HPV detection methods, geographic region of the study, total number of cases analyzed, HPV genotypes analyzed and/or detected, number testing HPV-positive, percent of HPV positivity, authors, and publication year (see Supporting Table 1 in the online version of this article). This list of items represents a modification of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statements,[24] adopted specifically for this purpose, where event rates represent the effect size recorded for each original study.

The authors divided HPV detection methods into two categories: PCR and non-PCR methods. The non-PCR methods comprises the following methods: immunohistochemistry (IHC), Southern blot hybridization (SB), dot blot hybridization (DB), filter in situ hybridization (FISH), hybrid capture II (HCII), in situ hybridization (ISH), non-isotopic in situ hybridization (NISH), and chromogene in situ hybridization (CISH). When more than one detection method was used, that particular study was categorized into the PCR group whenever PCR was included. In this case, only the PCR results were used in calculations. According to their geographic location, the studies were divided into the following groups: North America, Central and South America, Europe, China, other Asia, and Pacific, Africa/Middle East, and international (data from several regions).

Statistical Analyses

A specific software Comprehensive Meta Analysis (version 2.2.064; Biostat Inc., Englewood, NJ), was used to perform the meta-analysis. The software calculates the event rates based on the events and sample size data. To assess overall heterogeneity in event rates between the different studies, Cochran's Q homogeneity P value as well as I^2 statistics (for percentage of variation) were used.[25] To explore the eventual publication bias, funnel plots were drawn by plotting the logit event rates by their precision (1/SE),[26] evaluated for asymmetry using the following statistics: 1) Begg and Mazumdar rank correlation,[27] 2) Egger's test of the intercept (regression),[28] and 3) Duval and Tweedie's "trim and fill" method,[29] which imputes the results that are hypothetically missing due to publication bias.

TABLE I.
Meta-analysis of the 179 Studies on Laryngeal Squamous Cell Carcinoma Stratified by the Human Papillomavirus Detection Method.

				Point Estimates of Event Rates (Fixed-Effects Model)		Point Estimates of Event Rates (Random-Effects Model)				
HPV Detection Method	No. of Studies	Events	Sample Size	Point Estimate	95% CI	Point Estimate	95% CI	Homogeneity (Cochran's Q)*	I^{2*}	Homogeneity (*P* Value)*
PCR	127	1,387	5,161	0.311	0.297 to 0.325	0.271	0.241 to 0.302	564.857	77.693	.0001
Non-PCR	52	443	2,186	0.243	0.223 to 0.265	0.271	0.215 to 0.335	256.771	79.748	.0001
Summary	179	1,830	7,347	0.292	0.281 to 0.304	0.269	0.242 to 0.297	846.783	78.861	.0001
Total within (FE)								821.628		.0001
Total between (FE)								25.155		.0001
Total between (RE)								0.0000		.985

*Only calculated for fixed-effects model.
CI = confidence interval; FE = fixed-effects model; PCR = polymerase chain reaction; RE = random-effects model.

To assess the variation in event rates due to the differences between individual studies, we evaluated the study characteristics using stratified meta-analyses and restricted maximum likelihood meta-regression. Meta-regression formally compares these differences in event rates across the selected study-level covariates and estimates the among-study variance.[30] Because of inherent differences in sensitivity between the different HPV detection methods (PCR and non-PCR), meta-analyses were performed across these strata. Similarly, to distinguish true study-specific effects from random variation, all analyses were also stratified by the geographic regions of their origin, another potential source for variability in HPV prevalence. These two study characteristics (detection method and geographic location) were also tested as study-level covariates in meta-regression.

Sensitivity analysis was performed to assess the influence of each individual study on the strength and stability of the meta-analytic results, using the one-by-one study removal and evaluated by descriptively comparing the magnitude and precision of the random-effects summary event rates.

RESULTS

Eligible Studies

A total of 179 studies (see Supporting Table 1 in the online version of this article) were considered eligible for the present analysis,[3,4,9,16,19,31–204] comprising a sample size of 7,347 LSCCs with 1,830 events (approximately 25% of HPV positivity for all methods). In addition, some studies on HPV prevalence in recurrent respiratory papillomatosis (RRP), juvenile-onset papillomas, and adult-onset papillomas were included as far as they included also LSCC cases (metachronous or derived from RRP). A total of 5,161 samples were analyzed with PCR including 1,387 events (26.8% HPV positivity), whereas 2,186 samples were examined by non-PCR methods, with 443 events (20.3% HPV positivity) (Table I). The methods used to evaluate the HPV involvement include the following: PCR and non-PCR methods (IHC, DB, SB, ISH, FISH, NISH, HCII, and CISH). In studies using more than one technique, PCR results were computed whenever available[3,4,16,19,42,46,48,51,52,54,56–59,61,62,64–69,72,74–77,79,81–87,91,92,94–98,100,101,103–106,108–111,113–136,138–144,146–149,151–153,156–163,166–173,175–184,185,187,190,191,195–197,200–202] Conversely, when PCR was not used or its results were not available, the study was included in the non-PCR method group.[9,31–41,43–45,47,49,50,53,55,60,63,70,71,73,78,80,88–90,93,99,102,107,112,137,145,150,154,155,164,165,174,185,188,189,192–194,198,199,203,204]

Analytical Results

Point estimates of event rates. The crude HPV-positivity (1,830/7,347) translates to event rates of 0.292 (95% confidence interval [CI] 0.281 to 0.304) using the fixed-effects (FE) model, and 0.269 (95% CI: 0.242 to 0.297) using the random-effects (RE) model. There is a significant heterogeneity between the studies using PCR and non-PCR techniques (Cochran's Q statistics, P = .0001) (Table I). This also applies to overall comparison within strata (P = .0001), but not for between strata (P = .985). The percentage of variation (I^2) is highest (79.7%) for non–PCR-based studies. Using the RE model, both PCR and non-PCR techniques give exactly the same point estimates of HPV prevalence (0.271, 27.1%).

There is significant heterogeneity between the studies from different regions (P = .0001), with a variation of 68.9% (North America) to 78% (Europe) (Table II). The highest effect size (39.9%) is found in studies derived from China, followed by those conducted in Central and South America (29%) and Europe (27.9%). The between-strata comparison (RE model) indicates that the heterogeneity between the studies from different geographic regions is statistically significant (P = .0001).

Meta-regression. In meta-regression for HPV methods, the PCR method (n = 127) resulted in a point estimate that was significantly different (P = .0001) from the non-PCR methods (n = 52, reference), even though the HPV detection method was not a significant study-level covariate (P = .876) in meta-regression (Table III).

In meta-regression for geographic location, all locations except Europe (P = .023) resulted in point estimates that were significantly different from the reference (North America). However, in meta-regression, the geographic origin of the study did not have a significant impact on the effect size (P = .234) (Table IV).

TABLE II.
Meta-analysis of the 179 Studies on Laryngeal Squamous Cell Carcinoma Stratified by Their Geographic Area.

				Point Estimates of Event Rates (Fixed-Effects Model)		Point Estimates of Event Rates (Random-Effects Model)				
HPV Detection Method	No. of Studies	Events	Sample Size	Point Estimate	95% CI	Point Estimate	95% CI	Homogeneity (Cochran's Q)*	I^2*	Homogeneity (*P* Value)*
North America	45	370	1,652	0.236	0.214 to 0.259	0.246	0.201 to 0.298	141.779	68.966	.0001
Central and South America	14	190	640	0.324	0.286 to 0.364	0.290	0.217 to 0.377	57.424	77.361	.0001
Europe	82	828	3,266	0.308	0.290 to 0.327	0.279	0.238 to 0.323	371.602	78.202	.0001
Other Asia and Pacific	19	131	931	0.178	0.152 to 0.209	0.168	0.116 to 0.237	71.601	74.861	.0001
China	17	307	770	0.415	0.378 to 0.452	0.399	0.323 to 0.479	68.830	75.301	.0001
Africa and ME†	1	1	10							
Intercontinental†	1	3	78							
Summary	179	1,830	7,347	0.292	0.281 to 0.304	0.269	0.242 to 0.297	846.783	78.861	.0001
Total within (FE)								711.236		.0001
Total between (FE)								135.547		.0001
Total between (RE)								25.2057		.0001

*Only calculated for fixed-effects model.
†Only one study, meta-analysis redundant (omitted from the summary effect analysis).
CI = confidence interval; FE = fixed effects; RE = random effects.

Publication bias. There was no evidence for publication bias among studies based on PCR. For studies using non-PCR techniques, there was some evidence of publication bias. Duval and Tweedie's trim-and-fill method imputed three hypothetically missing studies, with a seemingly sizeable effect on adjusted point estimates (from 0.201 to 0.316), but this difference was not statistically significant as tested by Begg ($P = .138$) and Egger's ($P = .114$) tests.

There was no evidence of publication bias among studies from Central and South America, China, other Asia and Pacific, and from Europe. For studies from North America, the Duval and Tweedie's trim-and-fill method imputed two hypothetically missing studies, with a slight effect on adjusted point estimates (from 0.191 to 0.289), which was not statistically significant: Begg ($P = .071$) and Egger's ($P = .134$).

Sensitivity analysis. Meta-analytic results seemed robust to all (n = 179) one-by-one study removals, with no change in the magnitude and precision of the FE and RE summary point estimates of the effect size.

DISCUSSION

This meta-analysis with formal meta-analysis and meta-regression was performed to assess whether the wide variation in HPV prevalence in LSCC is due to different detection techniques as reported earlier.[3,5,15,23] There is little doubt that a marked heterogeneity exists between the studies based on PCR and non-PCR methods using the Q test and I^2 index (Table I). This marked heterogeneity justifies the adoption of the RE model to analyze the summary statistics.[25,205–209] Using the RE model, the most important conclusion implicates that there is no true heterogeneity between studies using different HPV detection techniques, as indicated by the nonsignificant homogeneity P value ($P = .985$) for the between-study comparison. Thus, we can revisit the concept raised in several studies suggesting that the differences in HPV prevalence in LSCC would be explained by different HPV detection techniques (intermethod variations)[5] or due to differences in detection criteria in the same method (intramethod variations).[47,210,211]

TABLE III.
Effect of HPV Detection Method on the Effect Size in Maximum Likelihood Meta-regression.

Study-Level Covariates		Effect Size†		Difference in Effect Size Estimates‡		
HPV Detection Method	No. of Studies (Homogeneity *P* Value)*	Point Estimate	95% CI	Difference in Point Estimates	95% CI	*P* Value
Non-PCR	52 (.0001)	0.271	0.215 to 0.335	1.000		Ref
PCR	127 (.0001)	0.271	0.241 to 0.302	0.065	0.044 to 0.086	.0001
Meta-regression for both methods	Slope: 0.028 (95% CI: −0.323 to 0.379) ($P = .876$); intercept: −1.044 (95% CI: −1.510 to −0.578) ($P < .001$)					

Slope: effect parameter (= regression coefficient β_1); intercept (= coefficient β_0).
*Cochran's Q.
†Random-effects model.
‡Calculated using Stata.
CI = confidence interval; HPV = human papillomavirus; PCR = polymerase chain reaction.

TABLE IV.
Effect of Geographic Origin of the Study on the Effect Size in Maximum Likelihood Meta-regression.

Study-Level Covariates, Geographic Origin of Study	No. of Studies (Homogeneity P Value)*	Effect Size† Point Estimate	Effect Size† 95% CI	Difference in Effect Size Estimates‡ Difference in Point Estimates	Difference in Effect Size Estimates‡ 95% CI	P Value
North America	45 (.0001)	0.246	0.201 to 0.298	1.000		Ref
Central and South America	14 (.0001)	0.290	0.217 to 0.377	0.072	0.032 to 0.113	.0001
Europe	82 (.0001)	0.279	0.238 to 0.323	0.029	0.004 to 0.054	.023
Other Asia and Pacific	19 (.0001)	0.168	0.116 to 0.237	−0.083	−0.113 to −0.053	.0001
China	17 (.0001)	0.399	0.323 to 0.479	0.175	0.135 to 0.215	.0001
Africa/ME§			1			
Intercontinental§			1			
Meta-regression for all	Slope: 0.072 (95% CI: −0.046 to 0.191) (P = .234); intercept: −1.191 (95% CI: −1.555 to −0.827) ($P < .001$)					

Slope: effect parameter (= regression coefficient β_1), intercept (= coefficient β_0)
*Cochran's Q.
†Random-effects model.
‡Calculated using Stata.
§Studies omitted from calculations.
CI = confidence interval.

The other study-level covariate was the geographic origin of the study, also listed as a potential cause of variation in HPV prevalence.[6,15,23] To validate this concept, we performed a meta-analysis stratified by the geographic area covered by the study (Table II). Both the Q test and the I^2 index demonstrated a marked heterogeneity between the studies within all different geographic areas, irrespective the number of studies included in each stratum. Both the within-strata and between-strata comparisons were significant, confirming the substantial heterogeneity between 1) the studies analyzing LSCC in the same geographic area and between 2) the studies assessing LSCC from different geographic areas. In addition, when the RE model was used to interpret the summary statistics, a significant (P = .0001) homogeneity P value was obtained for the between-strata comparison. This implicated that this wide variation (16.8% to 39.9%) in HPV prevalence from different geographic regions is significant according to the strict meta-analytical criteria. Table II depicts that the extreme of variation occurred in geographic areas with intermediate number of studies, whereas the summary effect size is within a rather narrow range (24.6%–27.9%) in regions with larger number of studies such as Europe and North America, emphasizing the need for meta-regression.

We also performed meta-regression to formally compare these differences in summary effect sizes.[29] In meta-regression with the HPV detection method as the covariate, the regression coefficient for the effect parameter (β1, or slope) was not statistically significant (P = .876) (Table III). The same was true when the geographic area of the study (P = .234) (Table IV), was tested for its impact as a study-level covariate. These data formally confirmed that HPV detection method and geographic area of the study are not significant study-level covariates accounting for the heterogeneity of the summary effect size estimates in LSCC studies.

Some evidence for publication bias was detected, but this usually had an insignificant effect on the adjusted point estimates in the stratified meta-analysis. Most importantly, there was no evidence on publication bias among PCR-based studies, and the slight publication bias among the non–PCR-based studies resulted in an insignificant change (Begg test, P = .138; Egger test, P = .114) of the point estimates (20.1%–31.6%). As to the geographic areas, some publication bias was evident only for studies from North America, with a slight impact on summary point estimates (19.1%–28.9%), which also proved to be nonsignificant (Begg test, P = .071; Egger test, P = .134).

One of the potential limitations of the present meta-analysis is associated with the shortcomings of the Q statistic, known to have a poor power to detect true heterogeneity in meta-analysis including a small number of studies, but excessive power to detect even insignificant variability when a large number of studies is available.[25,205–209] This can be a potential weakness only when the meta-analysis is stratified by HPV detection method and geographic areas of the study, which inevitably leads to strata with limited number of studies. This, however, is compensated by the I^2 index, because the indices obtained from meta-analyses with different numbers of studies and different effect metrics are directly comparable.[209]

Taken together, this meta-analysis based on 179 eligible studies on HPV detection in LSCC implicates that HPV prevalence in LSCC was 25%, and it varies according to 1) HPV detection method and 2) geographic origin. However, in stratified meta-analysis, this variability reaches statistical significance only for geographic area of the study. In formal meta-regression, however, none of these study characteristics are significant study-level covariates accounting for the heterogeneity of HPV prevalence. Finally, it is important to note that this study does not analyze the physical state or transcriptional activity of HPV in these tumors. Multicentric follow-up studies are needed to further characterize the role of HPV in LSCC.

CONCLUSION

In stratified meta-analysis, variability in HPV detection rates in LSCC is explained by geographic area of the studies and not by HPV detection method. None of the two were significant study-level covariates in formal meta-regression.

BIBLIOGRAPHY

1. Syrjanen K, Syrjanen S, Lamberg M, Pyrhonen S, Nuutinen J. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. *Int J Oral Surg* 1983;12:418–424.
2. Syrjanen S. HPV infections and tonsillar carcinoma. *J Clin Pathol* 2004; 57:449–455.
3. Wei W, Shi Q, Guo F, Zhang BY, Chen C, et al. The distribution of human papillomavirus in tissues from patients with head and neck squamous cell carcinoma. *Oncol Rep* 2012;28:1750–1756.
4. Sethi S, Ali-Fehmi R, Franceschi S, et al. Characteristics and survival of head and neck cancer by HPV status: a cancer registry-based study. *Int J Cancer* 2012;131:1179–1186.
5. Liu H, Li J, Diao M, Cai Z, Yang J, Zeng Y. Statistical analysis of human papillomavirus in a subset of upper aerodigestive tract tumors. *J Med Virol* 2013;85:1775–1785.
6. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2005;14:467–475.
7. Begum S, Cao D, Gillison M, Zahurak M, Westra WH. Tissue distribution of human papillomavirus 16 DNA integration in patients with tonsillar carcinoma. *Clin Cancer Res* 2005;11:5694–5699.
8. Singhi AD, Westra WH. Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus-associated head and neck cancer based on prospective clinical experience. *Cancer* 2010;116:2166–2173.
9. Lewis JS Jr, Ukpo OC, Ma XJ, et al. Transcriptionally-active high-risk human papillomavirus is rare in oral cavity and laryngeal/hypopharyngeal squamous cell carcinomas—a tissue microarray study utilizing E6/E7 mRNA in situ hybridization. *Histopathology* 2012;60:982–991.
10. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011;61:69–90.
11. National Cancer Institute. Surveillance, Epidemiology and End Results Program (SEER database). Available at: http://www.seer.cancer.gov. Accessed May 10, 2015.
12. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010;127:2893–2917.
13. Ndiaye C, Mena M, Alemany L, et al. HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. *Lancet Oncol* 2014;15:1319–1331.
14. Li X, Gao L, Li H, et al. Human papillomavirus infection and laryngeal cancer risk: a systematic review and meta-analysis. *J Infect Dis* 2013; 207:479–488.
15. Zhang C, Deng Z, Chen Y, Suzuki M, Xie M. Is there a higher prevalence of human papillomavirus infection in Chinese laryngeal cancer patients? A systematic review and meta-analysis [published online October 26, 2014]. *Eur Arch Otorhinolaryngol* doi: 10.1007/s00405-014-3345-3.
16. Chernock RD, Wang X, Gao G, et al. Detection and significance of human papillomavirus, CDKN2A(p16) and CDKN1A(p21) expression in squamous cell carcinoma of the larynx. *Mod Pathol* 2013;26:223–231.
17. Bishop JA, Lewis Jr JS, Rocco JW, Faquin WC. HPV-related squamous cell carcinoma of the head and neck: an update on testing in routine pathology practice. *Semin Diagn Pathol* 2015;32:344–351.
18. Smeets SJ, Hesselink AT, Speel EJ, et al. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. *Int J Cancer* 2007;121:2465–2472.
19. Halec G, Holzinger D, Schmitt M, et al. Biological evidence for a causal role of HPV16 in a small fraction of laryngeal squamous cell carcinoma. *Br J Cancer* 2013;109:172–183.
20. Gao N, Li Y, Li LJ, Wen YM. Clinical analysis of head and neck cancer cases in south-west China 1953–2002. *J Int Med Res* 2009;37:189–197.
21. Li W, Tran N, Lee SC, et al. New evidence for geographic variation in the role of human papillomavirus in tonsillar carcinogenesis. *Pathology* 2007;39:217–222.
22. Jiron J, Sethi S, Ali-Fehmi R, et al. Racial disparities in Human Papillomavirus (HPV) associated head and neck cancer. *Am J Otolaryngol* 2014;35:147–153.
23. Xu Y, Liu S, Yi H, et al. Human papillomavirus infection in 674 Chinese patients with laryngeal squamous cell carcinoma. *PLoS One* 2014;9:e115914.
24. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 2009;339:b2535.
25. Hardy RJ, Thompson SG. Detecting and describing heterogeneity in meta-analysis. *Stat Med* 1998;17:841–856.
26. Sterne JA, Gavaghan D, Egger M. Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. *J Clin Epidemiol* 2000;53:1119–1129.
27. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;50:1088–1101.
28. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629–634.
29. Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000;56:455–463.
30. Thompson SG, Sharp SJ. Explaining heterogeneity in meta-analysis: a comparison of methods. *Stat Med* 1999;18:2693–2708.
31. Syrjanen K, Syrjanen S, Pyrhonen S. Human papilloma virus (HPV) antigens in lesions of laryngeal squamous cell carcinomas. *ORL J Otorhinolaryngol Relat Spec* 1982;44:323–334.
32. Abramson AL, Brandsma J, Steinberg B, Winkler B. Verrucous carcinoma of the larynx. Possible human papillomavirus etiology. *Arch Otolaryngol* 1985;111:709–715.
33. Brandsma JL, Steinberg BM, Abramson AL, Winkler B. Presence of human papillomavirus type 16 related sequences in verrucous carcinoma of the larynx. *Cancer Res* 1986;46(4 pt 2):2185–2188.
34. Kahn T, Schwarz E, zur Hausen H. Molecular cloning and characterization of the DNA of a new human papillomavirus (HPV 30) from a laryngeal carcinoma. *Int J Cancer* 1986;37:61–65.
35. Scheurlen W, Stremlau A, Gissmann L, Hohn D, Zenner HP, zur Hausen H. Rearranged HPV 16 molecules in an anal and in a laryngeal carcinoma. *Int J Cancer* 1986;38:671–676.
36. Stremlau A, Zenner HP, Gissmann L, zur Hausen H.. Demonstration and organizational structure of the DNA of human papillomaviruses in laryngeal and hypopharyngeal carcinomas [in German]. *Laryngol Rhinol Otol (Stuttg)* 1987;66:311–315.
37. Loning T, Meichsner M, Milde-Langosch K, et al. HPV DNA detection in tumours of the head and neck: a comparative light microscopy and DNA hybridization study. *ORL J Otorhinolaryngol Relat Spec* 1987;49:259–269.
38. Syrjanen S, Syrjanen K, Mantyjarvi R, Collan Y, Karja J. Human papillomavirus DNA in squamous cell carcinomas of the larynx demonstrated by in situ DNA hybridization. *ORL J Otorhinolaryngol Relat Spec* 1987; 49:175–186.
39. Zarod AP, Rutherford JD, Corbitt G. Malignant progression of laryngeal papilloma associated with human papilloma virus type 6 (HPV-6) DNA. *J Clin Pathol* 1988;41:280–283.
40. Kashima H, Wu TC, Mounts P, Heffner D, Cachay A, Hyams V. Carcinoma ex-papilloma: histologic and virologic studies in whole-organ sections of the larynx. *Laryngoscope* 1988;98(6 pt 1):619–624.
41. Brandsma JL, Abramson AL. Association of papillomavirus with cancers of the head and neck. *Arch Otolaryngol Head Neck Surg* 1989;115:621–625.
42. Kiyabu MT, Shibata D, Arnheim N, Martin WJ, Fitzgibbons PL. Detection of human papillomavirus in formalin-fixed, invasive squamous carcinomas using the polymerase chain reaction. *Am J Surg Pathol* 1989;13: 221–224.
43. Ward P, Mounts P. Heterogeneity in mRNA of human papillomavirus type-6 subtypes in respiratory tract lesions. *Virology* 1989;168:1–12.
44. Lindeberg H, Syrjanen S, Karja J, Syrjanen K. Human papillomavirus type 11 DNA in squamous cell carcinomas and pre-existing multiple laryngeal papillomas. *Acta Otolaryngol* 1989;107:141–149.
45. Ishibashi T, Matsushima S, Tsunokawa Y, et al. Human papillomavirus DNA in squamous cell carcinoma of the upper aerodigestive tract. *Arch Otolaryngol Head Neck Surg* 1990;116:294–298.
46. Hoshikawa T, Nakajima T, Uhara H, et al. Detection of human papillomavirus DNA in laryngeal squamous cell carcinomas by polymerase chain reaction. *Laryngoscope* 1990;100:647–650.
47. Kulski JK, Demeter T, Mutavdzic S, Sterrett GF, Mitchell KM, Pixley EC. Survey of histologic specimens of human cancer for human papillomavirus types 6/11/16/18 by filter in situ hybridization. *Am J Clin Pathol* 1990;94:566–570.
48. Perez-Ayala M, Ruiz-Cabello F, Esteban F, et al. Presence of HPV 16 sequences in laryngeal carcinomas. *Int J Cancer* 1990;46:8–11.
49. Vonka R, Vesely J, Sibl O, Brichacek B. The presence of human papillomavirus deoxyribonucleic acid in tumors in the otorhinolaryngologic area [in Czech]. *Cas Lek Cesk* 1990;129:334–336.
50. McCullough DW, McNicol PJ. Laryngeal carcinoma associated with human papillomavirus type 16. *J Otolaryngol* 1991;20:97–99.
51. Morgan DW, Abdullah V, Quiney R, Myint S. Human papilloma virus and carcinoma of the laryngopharynx. *J Laryngol Otol* 1991;105:288–290.
52. Ogura H, Watanabe S, Fukushima K, Masuda Y, Fujiwara T, Yabe Y. Presence of human papillomavirus type 18 DNA in a pharyngeal and a laryngeal carcinoma. *Jpn J Cancer Res* 1991;82:1184–1186.
53. Wang H, Lin YC, Kang XS, et al. Detection of human papilloma virus (HPV) in laryngeal carcinoma tissue. *Chin Med J (Engl)* 1991;104:523–525.
54. Watts SL, Brewer EE, Fry TL. Human papillomavirus DNA types in squamous cell carcinomas of the head and neck. *Oral Surg Oral Med Oral Pathol* 1991;71:701–707.
55. Arndt O, Zeise K, Bauer I, Brock J. Type 6/11 and 16/18 squamous epithelial cancers of the upper respiratory tract and digestive system. An in situ hybridization study [in German]. *Laryngorhinootologie* 1992;71: 500–504.
56. Yao CD. Detection of HPV-16-related DNA sequence in laryngeal squamous cell carcinoma [in Chinese]. *Zhonghua Er Bi Yan Hou Ke Za Zhi* 1992;27:241–242, 256.

57. Anwar K, Nakakuki K, Naiki H, Inuzuka M. ras gene mutations and HPV infection are common in human laryngeal carcinoma. *Int J Cancer* 1993; 53:22–28.
58. Brandwein MS, Nuovo GJ, Biller H. Analysis of prevalence of human papillomavirus in laryngeal carcinomas. Study of 40 cases using polymerase chain reaction and consensus primers. *Ann Otol Rhinol Laryngol* 1993; 102(4 pt 1):309–313.
59. Kasperbauer JL, O'Halloran GL, Espy MJ, Smith TF, Lewis JE. Polymerase chain reaction (PCR) identification of human papillomavirus (HPV) DNA in verrucous carcinoma of the larynx. *Laryngoscope* 1993;103(4 pt 1):416–420.
60. Makowska W, Rogozinski T, Zawadowski J, Waloryszak B. Human papilloma virus infection in the case of larynx carcinoma [in Polish]. *Otolaryngol Pol* 1993;47:23–25.
61. Ogura H, Watanabe S, Fukushima K, Masuda Y, Fujiwara T, Yabe Y. Human papillomavirus DNA in squamous cell carcinomas of the respiratory and upper digestive tracts. *Jpn J Clin Oncol* 1993;23:221–225.
62. Tyan YS, Liu ST, Ong WR, Chen ML, Shu CH, Chang YS. Detection of Epstein-Barr virus and human papillomavirus in head and neck tumors. *J Clin Microbiol* 1993;31:53–56.
63. van Rensburg EJ, Venter EH, Simson IW. Human papillomavirus DNA in aerodigestive squamous carcinomas demonstrated by means of in situ hybridisation. *S Afr Med J* 1993;83:516–518.
64. Arndt O, Brock J, Kundt G, Mullender A. Detection of human papillomavirus DNA in formalin fixed invasive squamous cell carcinoma of the larynx with polymerase chain reaction (PCR) [in German]. *Laryngorhinootologie* 1994;73:527–532.
65. Doyle DJ, Henderson LA, LeJeune FE Jr, Miller RH. Changes in human papillomavirus typing of recurrent respiratory papillomatosis progressing to malignant neoplasm. *Arch Otolaryngol Head Neck Surg* 1994;120: 1273–1276.
66. Clayman GL, Stewart MG, Weber RS, El-Naggar AK, Grimm EA. Human papillomavirus in laryngeal and hypopharyngeal carcinomas. Relationship to survival. *Arch Otolaryngol Head Neck Surg* 1994;120:743–748.
67. Fliss DM, Noble-Topham SE, McLachlin M, et al. Laryngeal verrucous carcinoma: a clinicopathologic study and detection of human papillomavirus using polymerase chain reaction. *Laryngoscope* 1994;104:146–152.
68. Gorgoulis V, Rassidakis G, Karameris A, Giatromanolaki A, Barbatis C, Kittas C. Expression of p53 protein in laryngeal squamous cell carcinoma and dysplasia: possible correlation with human papillomavirus infection and clinicopathological findings. *Virchows Arch* 1994;425:481–489.
69. Lewensohn-Fuchs I, Munck-Wikland E, Berke Z, et al. Involvement of aberrant p53 expression and human papillomavirus in carcinoma of the head and neck and esophagus. *Anticancer Res* 1994;14:1281–1285.
70. Li Y, Witte D, Myer C, et al. Involvement of episomal hpv31 in a laryngeal carcinoma—persistent episomal maintenance of HPV DNA after passage through nude-mice. *Int J Oncol* 1994;4:1377–1382.
71. Multhaupt HA, Fessler JN, Warhol MJ. Detection of human papillomavirus in laryngeal lesions by in situ hybridization. *Hum Pathol* 1994;25: 1302–1305.
72. Shidara K, Suzuki T, Hara F, Nakajima T. Lack of synergistic association between human papillomavirus and ras gene point mutation in laryngeal carcinomas. *Laryngoscope* 1994;104(8 pt 1):1008–1012.
73. Simon M, Kahn T, Schneider A, Pirsig W. Laryngeal carcinoma in a 12-year-old child. Association with human papillomavirus 18 and 33. *Arch Otolaryngol Head Neck Surg* 1994;120:277–282.
74. Salam MA, Rockett J, Morris A. General primer-mediated polymerase chain reaction for simultaneous detection and typing of human papillomavirus DNA in laryngeal squamous cell carcinomas. *Clin Otolaryngol Allied Sci* 1995;20:84–88.
75. Fouret P, Martin F, Flahault A, Saint-Guily JL. Human papillomavirus infection in the malignant and premalignant head and neck epithelium. *Diagn Mol Pathol* 1995;4:122–127.
76. Almadori G, Cadoni G, Cattani P, et al. Detection of human papillomavirus DNA in laryngeal squamous cell carcinoma by polymerase chain reaction. *Eur J Cancer* 1996;32A:783–788.
77. Alvarez Alvarez I, Sánchez Lazo P, Ramos González S, Rodrigo Tapia JP, Llorente Pendas JL, Suárez Nieto C. Simultaneous screening of HPV-6b and 16 in pharyngolaryngeal cancer [in Spanish]. *Acta Otorrinolaringol Esp* 1996;47:93–96.
78. Cerovac Z, Sarcevic B, Kralj Z, Ban J. Detection of human papillomavirus (HPV) type 6, 16 and 18 in head and neck squamous cell carcinomas by in situ hybridization. *Neoplasma* 1996;43:185–194.
79. Lie ES, Karlsen F, Holm R. Presence of human papillomavirus in squamous cell laryngeal carcinomas. A study of thirty-nine cases using polymerase chain reaction and in situ hybridization. *Acta Otolaryngol* 1996; 116:900–905.
80. Lopez-Amado M, Garcia-Caballero T, Lozano-Ramirez A, Labella-Caballero T. Human papillomavirus and p53 oncoprotein in verrucous carcinoma of the larynx. *J Laryngol Otol* 1996;110:742–747.
81. Shen J, Tate JE, Crum CP, Goodman ML. Prevalence of human papillomaviruses (HPV) in benign and malignant tumors of the upper respiratory tract. *Mod Pathol* 1996;9:15–20.
82. Czegledy J, Major T, Juhasz A, Repassy G, Gergely L. Detection of human papillomavirus gene sequences in laryngeal tumors and premalignant changes by polymerase chain reaction [in Hungarian]. *Orv Hetil* 1997; 138:1891–1895.
83. Paz IB, Cook N, Odom-Maryon T, Xie Y, Wilczynski SP. Human papillomavirus (HPV) in head and neck cancer. An association of HPV 16 with squamous cell carcinoma of Waldeyer's tonsillar ring. *Cancer* 1997;79: 595–604.
84. Vowles RH, Myint S, Croft CB. The incidence and detection of HPV in the upper aerodigestive tract using brush and biopsy techniques. *J Laryngol Otol* 1997;111:215–217.
85. Xie M, Xiao J, Tao Z, Luo J. A preliminary study on p53 gene expression and infection of human papilloma virus in laryngeal squamous cell carcinoma [in Chinese]. *Hunan Yi Ke Da Xue Xue Bao* 1997;22:209–211.
86. Alvarez Alvarez I, Sanchez Lazo P, Ramos Gonzalez S, Rodrigo Tapia JP, Nuñez Batalla F, Suarez Nieto C. Using polymerase chain reaction to human papillomavirus in oral and pharyngolaryngeal carcinomas. *Am J Otolaryngol* 1997;18:375–381.
87. Atula S, Auvinen E, Grenman R, Syrjanen S. Human papillomavirus and Epstein-Barr virus in epithelial carcinomas of the head and neck region. *Anticancer Res* 1997;17:4427–4433.
88. Caruso ML, Valentini AM. Localization of p53 protein and human papillomavirus in laryngeal squamous lesions. *Anticancer Res* 1997;17:4671–4675.
89. Qin Z, Dong M. Study on HPV infection and p53 protein expression in laryngeal carcinoma [in Chinese]. *Lin Chuang Er Bi Yan Hou Ke Za Zhi* 1997;11:546–549.
90. Ren J, Wang X, Zhu Q. Study on the relation between HPV and tumors of the throat and larynx [in Chinese]. *Lin Chuang Er Bi Yan Hou Ke Za Zhi* 1997;11:157–159.
91. Cattani P, Hohaus S, Bellacosa A, et al. Association between cyclin D1 (CCND1) gene amplification and human papillomavirus infection in human laryngeal squamous cell carcinoma. *Clin Cancer Res* 1998;4: 2585–2589.
92. Garcia-Milian R, Hernandez H, Panade L, et al. Detection and typing of human papillomavirus DNA in benign and malignant tumours of laryngeal epithelium. *Acta Otolaryngol* 1998;118:754–758.
93. Kim JY, Cho KJ, Lee SS, Khang SK, Shim YS. Clinicopathologic study of basaloid squamous carcinoma of the upper aerodigestive tract. *J Korean Med Sci* 1998;13:269–274.
94. Ma XL, Ueno K, Pan ZM, Hi SZ, Ohyama M, Eizuru Y. Human papillomavirus DNA sequences and p53 over-expression in laryngeal squamous cell carcinomas in Northeast China. *J Med Virol* 1998;54:186–191.
95. Matzow T, Boysen M, Kalantari M, Johansson B, Hagmar B. Low detection rate of HPV in oral and laryngeal carcinomas. *Acta Oncol* 1998;37: 73–76.
96. Mineta H, Ogino T, Amano HM, et al. Human papilloma virus (HPV) type 16 and 18 detected in head and neck squamous cell carcinoma. *Anticancer Res* 1998;18:4765–4768.
97. Soto Y, Valdes C, Mune M, Pimentel T, Ramirez R. Detection of type 16 human papillomavirus DNA in formalin-fixed invasive squamous cells from laryngeal cancers by polymerase chain reaction. *Mem Inst Oswaldo Cruz* 1998;93:439–440.
98. Zhao S, Lu S, Fei S. Detection of human papillomavirus in Chinese laryngeal carcinoma with digoxigenin labelled probe prepared by PCR [in Chinese]. *Lin Chuang Er Bi Yan Hou Ke Za Zhi* 1998;12:153–155.
99. Atula S, Grenman R, Kujari H, Syrjanen S. Detection of human papillomavirus (HPV) in laryngeal carcinoma cell lines provides evidence for a heterogeneic cell population. *Eur J Cancer* 1999;35:825–832.
100. Gorgoulis VG, Zacharatos P, Kotsinas A, et al. Human papilloma virus (HPV) is possibly involved in laryngeal but not in lung carcinogenesis. *Hum Pathol* 1999;30:274–283.
101. Lindeberg H, Krogdahl A. Laryngeal cancer and human papillomavirus: HPV is absent in the majority of laryngeal carcinomas. *Cancer Lett* 1999;146:9–13.
102. Mizokami H, Sawatsubashi M, Tokunaga O, Shin T. Loss of retinoblastoma protein expression in laryngeal squamous cell carcinoma. *Mod Pathol* 1999;12:47–53.
103. Pintos J, Franco EL, Black MJ, Bergeron J, Arella M. Human papillomavirus and prognoses of patients with cancers of the upper aerodigestive tract. *Cancer* 1999;85:1903–1909.
104. Wang L, Zhang T, Zhang F. Study on the association between squamous cell carcinoma of larynx and HPV subgene by PCR [in Chinese]. *Zhonghua Er Bi Yan Hou Ke Za Zhi* 1999;34:106–107.
105. Zhao S, Ye Q, Lu S. Detection of human papillomavirus in laryngeal carcinoma with digoxigenin labelled probe prepared by polymerase chain reaction [in Chinese]. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 1999;13:13–16.
106. Badaracco G, Venuti A, Morello R, Muller A, Marcante ML. Human papillomavirus in head and neck carcinomas: prevalence, physical status and relationship with clinical/pathological parameters. *Anticancer Res* 2000;20:1301–1305.
107. Brito H, Vassallo J, Altemani A. Detection of human papillomavirus in laryngeal squamous dysplasia and carcinoma. An in situ hybridization and signal amplification study. *Acta Otolaryngol* 2000;120:540–544.
108. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000;92:709–720.
109. Smith EM, Summersgill KF, Allen J, et al. Human papillomavirus and risk of laryngeal cancer. *Ann Otol Rhinol Laryngol* 2000;109:1069–1076.
110. Venuti A, Manni V, Morello R, De MF, Marzetti F, Marcante ML. Physical state and expression of human papillomavirus in laryngeal carcinoma and surrounding normal mucosa. *J Med Virol* 2000;60:396–402.

111. Almadori G, Cadoni G, Cattani P, et al. Human papillomavirus infection and epidermal growth factor receptor expression in primary laryngeal squamous cell carcinoma. *Clin Cancer Res* 2001;7:3988–3993.
112. Kaya H, Kotiloglu E, Inanli S, et al. Prevalence of human papillomavirus (HPV) DNA in larynx and lung carcinomas. *Pathologica* 2001;93:531–534.
113. Klussmann JP, Weissenborn SJ, Wieland U, et al. Prevalence, distribution, and viral load of human papillomavirus 16 DNA in tonsillar carcinomas. *Cancer* 2001;92:2875–2884.
114. Li J, Yang J, Yi Z, Lin Y, Zhou A. Human papilloma virus infection and expression of p16 protein in laryngeal papilloma and laryngeal carcinoma [in Chinese]. *Zhonghua Er Bi Yan Hou Ke Za Zhi* 2001;36:51–54.
115. Makowska W, Malejczyk M, Kapiszewska D, Nyckowska J, Wojcikiewicz E, Wroblewska B. Human papillomavirus (HPV) in the laryngeal carcinoma [in Polish]. *Otolaryngol Pol* 2001;55:263–266.
116. Mork J, Lie AK, Glattre E, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2001;344:1125–1131.
117. Morshed K, Stenzel A, Szymanski M, et al. Detection of human papillomavirus type 16 and 18 in laryngeal cancer using PCR methods [in Polish]. *Otolaryngol Pol* 2001;55:29–33.
118. Mutiu A, Alexiu I, Chivu M, et al. Detection of human papillomavirus gene sequences in cell lines derived from laryngeal tumors. *J Cell Mol Med* 2001;5:49–59.
119. van Houten VM, Snijders PJ, van den Brekel MW, et al. Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. *Int J Cancer* 2001; 93:232–235.
120. Jacob SE, Sreevidya S, Chacko E, Pillai MR. Cellular manifestations of human papillomavirus infection in laryngeal tissues. *J Surg Oncol* 2002;79:142–150.
121. Lin S, Chen Z, Chen MH, Wang HM. Express of human papillomavirus, P53 and H-ras gene in laryngeal cancer [in Chinese]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2002;31:231–234.
122. Ringström E, Peters E, Hasegawa M, Posner M, Liu M, Kelsey KT. Human papillomavirus type 16 and squamous cell carcinoma of the head and neck. *Clin Cancer Res* 2002;8:3187–3192.
123. Szentirmay Z, Szanto I, Balint I, et al. Causal association between human papilloma virus infection and head and neck and esophageal squamous cell carcinoma [in Hungarian]. *Magy Onkol* 2002;46:35–41.
124. Du J, Chen GG, Vlantis AC, Xu H, Tsang RK, van Hasselt AC. The nuclear localization of NFkappaB and p53 is positively correlated with HPV16 E7 level in laryngeal squamous cell carcinoma. *J Histochem Cytochem* 2003;51:533–539.
125. El-Mofty SK, Lu DW. Prevalence of human papillomavirus type 16 DNA in squamous cell carcinoma of the palatine tonsil, and not the oral cavity, in young patients: a distinct clinicopathologic and molecular disease entity. *Am J Surg Pathol* 2003;27:1463–1470.
126. Fischer M, von WF. Evaluation and application of a broad-spectrum polymerase chain reaction assay for human papillomaviruses in the screening of squamous cell tumours of the head and neck. *Acta Otolaryngol* 2003;123:752–758.
127. Godlewska-Zoladkowska K, Olszewska E, Chodynicki S, Miasko A, Chyczewski L. Verrucous carcinoma—histological and molecular analysis [in Polish]. *Otolaryngol Pol* 2003;57:793–797.
128. Klussmann JP, Weissenborn SJ, Wieland U, et al. Human papillomavirus-positive tonsillar carcinomas: a different tumor entity? *Med Microbiol Immunol* 2003;192:129–132.
129. Azzimonti B, Pagano M, Mondini M, et al. Altered patterns of the interferon-inducible gene IFI16 expression in head and neck squamous cell carcinoma: immunohistochemical study including correlation with retinoblastoma protein, human papillomavirus infection and proliferation index. *Histopathology* 2004;45:560–572.
130. Baez A, Almodovar JI, Cantor A, et al. High frequency of HPV16-associated head and neck squamous cell carcinoma in the Puerto Rican population. *Head Neck* 2004;26:778–784.
131. Kleist B, Bankau A, Lorenz G, Jager B, Poetsch M. Different risk factors in basaloid and common squamous head and neck cancer. *Laryngoscope* 2004;114:1063–1068.
132. Kumar RV, Shenoy AM, Daniel R, Shah KV. Cyclin D1, p53, MIB1, intratumoral microvessel density, and human papillomavirus in advanced laryngeal carcinoma: association with nodal metastasis. *Otolaryngol Head Neck Surg* 2004;131:509–513.
133. Reidy PM, Dedo HH, Rabah R, et al. Integration of human papillomavirus type 11 in recurrent respiratory papilloma-associated cancer. *Laryngoscope* 2004;114:1906–1909.
134. Hoffmann M, Gorogh T, Gottschlich S, et al. Human papillomaviruses in head and neck cancer: 8 year-survival-analysis of 73 patients. *Cancer Lett* 2005;218:199–206.
135. Koppikar P, deVilliers EM, Mulherkar R. Identification of human papillomaviruses in tumors of the oral cavity in an Indian community. *Int J Cancer* 2005;113:946–950.
136. Major T, Szarka K, Sziklai I, Gergely L, Czegledy J. The characteristics of human papillomavirus DNA in head and neck cancers and papillomas. *J Clin Pathol* 2005;58:51–55.
137. Morshed K, Korobowicz E, Szymanski M, Skomra D, Golabek W. Immunohistochemical demonstration of multiple HPV types in laryngeal squamous cell carcinoma. *Eur Arch Otorhinolaryngol* 2005;262:917–920.
138. Morshed K, Polz-Dacewicz M, Rajtar B, Szymanski M, Ziaja-Soltys M, Golabek W. The prevalence of E6/E7 HPV type 16 in laryngeal cancer and in normal mucosa [in Polish]. *Pol Merkur Lekarski* 2005;19:291–293.
139. Szladek G, Juhasz A, Kardos G, et al. High co-prevalence of genogroup 1 TT virus and human papillomavirus is associated with poor clinical outcome of laryngeal carcinoma. *J Clin Pathol* 2005;58:402–405.
140. Torrente MC, Ampuero S, Abud M, Ojeda JM. Molecular detection and typing of human papillomavirus in laryngeal carcinoma specimens. *Acta Otolaryngol* 2005;125:888–893.
141. Vlachtsis K, Nikolaou A, Markou K, Fountzilas G, Daniilidis I. Clinical and molecular prognostic factors in operable laryngeal cancer. *Eur Arch Otorhinolaryngol* 2005;262:890–898.
142. de Oliveira DE, Bacchi MM, Macarenco RS, Tagliarini JV, Cordeiro RC, Bacchi CE. Human papillomavirus and Epstein-Barr virus infection, p53 expression, and cellular proliferation in laryngeal carcinoma. *Am J Clin Pathol* 2006;126:284–293.
143. Manjarrez ME, Ocadiz R, Valle L, et al. Detection of human papillomavirus and relevant tumor suppressors and oncoproteins in laryngeal tumors. *Clin Cancer Res* 2006;12:6946–6951.
144. Slebos RJ, Yi Y, Ely K, et al. Gene expression differences associated with human papillomavirus status in head and neck squamous cell carcinoma. *Clin Cancer Res* 2006;12(3 pt 1):701–709.
145. Applebaum KM, Furniss CS, Zeka A, et al. Lack of association of alcohol and tobacco with HPV16-associated head and neck cancer. *J Natl Cancer Inst* 2007;99:1801–1810.
146. Badaracco G, Rizzo C, Mafera B, et al. Molecular analyses and prognostic relevance of HPV in head and neck tumours. *Oncol Rep* 2007;17:931–939.
147. Furniss CS, McClean MD, Smith JF, et al. Human papillomavirus 16 and head and neck squamous cell carcinoma. *Int J Cancer* 2007;120:2386–2392.
148. Gallegos-Hernandez JF, Paredes-Hernandez E, Flores-Diaz R, Minauro-Munoz G, Presa-Garcia T, Hernandez-Hernandez DM. Human papillomavirus: association with head and neck cancer [in Spanish]. *Cir Cir* 2007;75:151–155.
149. Gungor A, Cincik H, Baloglu H, Cekin E, Dogru S, Dursun E. Human papilloma virus prevalence in laryngeal squamous cell carcinoma. *J Laryngol Otol* 2007;121:772–774.
150. Konig F, Krekeler G, Honig JF, Cordon-Cardo C, Fischer G, Korabiowska M. Relation between human papillomavirus positivity and p16 expression in head and neck carcinomas—a tissue microarray study. *Anticancer Res* 2007;27:283–288.
151. Koskinen WJ, Brondbo K, Mellin DH, et al. Alcohol, smoking and human papillomavirus in laryngeal carcinoma: a Nordic prospective multicenter study. *J Cancer Res Clin Oncol* 2007;133:673–678.
152. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst* 2008;100:261–269.
153. Guvenc MG, Midilli K, Ozdogan A, et al. Detection of HHV-8 and HPV in laryngeal carcinoma. *Auris Nasus Larynx* 2008;35:357–362.
154. Laco J, Slaninka I, Jirasek M, Celakovsky P, Vosmikova H, Ryska A. High-risk human papillomavirus infection and p16INK4a protein expression in laryngeal lesions. *Pathol Res Pract* 2008;204:545–552.
155. Lee WT, Tubbs RR, Teker AM, et al. Use of in situ hybridization to detect human papillomavirus in head and neck squamous cell carcinoma patients without a history of alcohol or tobacco use. *Arch Pathol Lab Med* 2008;132:1653–1656.
156. Morshed K, Polz-Dacewicz M, Szymanski M, Polz D. Short-fragment PCR assay for highly sensitive broad-spectrum detection of human papillomaviruses in laryngeal squamous cell carcinoma and normal mucosa: clinico-pathological evaluation. *Eur Arch Otorhinolaryngol* 2008; 265(suppl 1):S89–S96.
157. Smith EM, Wang D, Rubenstein LM, Morris WA, Turek LP, Haugen TH. Association between p53 and human papillomavirus in head and neck cancer survival. *Cancer Epidemiol Biomarkers Prev* 2008;17:421–427.
158. Baumann JL, Cohen S, Evjen AN, et al. Human papillomavirus in early laryngeal carcinoma. *Laryngoscope* 2009;119:1531–1537.
159. Boscolo-Rizzo P, Da Mosto MC, Fuson R, Frayle-Salamanca H, Trevisan R, Del MA. HPV-16 E6 L83V variant in squamous cell carcinomas of the upper aerodigestive tract. *J Cancer Res Clin Oncol* 2009;135:559–566.
160. Bozdayi G, Kemaloglu Y, Ekinci O, et al. Role of human papillomavirus in the clinical and histopathologic features of laryngeal and hypopharyngeal cancers. *J Otolaryngol Head Neck Surg* 2009;38:119–125.
161. Gallo A, Degener AM, Pagliuca G, et al. Detection of human papillomavirus and adenovirus in benign and malignant lesions of the larynx. *Otolaryngol Head Neck Surg* 2009;141:276–281.
162. Miranda FA, Hassumi MK, Guimaraes MC, et al. Galectin-3 overexpression in invasive laryngeal carcinoma, assessed by computer-assisted analysis. *J Histochem Cytochem* 2009;57:665–673.
163. Moyano S, Ordi J, Caballero M, et al. Laryngeal squamous cell carcinoma in HIV-positive patients: lack of association with human papillomavirus infection. *HIV Med* 2009;10:634–639.
164. Chernock RD, Lewis JS Jr, Zhang Q, El-Mofty SK. Human papillomavirus-positive basaloid squamous cell carcinomas of the upper aerodigestive tract: a distinct clinicopathologic and molecular subtype of basaloid squamous cell carcinoma. *Hum Pathol* 2010;41:1016–1023.

165. Chitose S, Sakazaki T, Ono T, Kurita T, Mihashi H, Nakashima T. Immunological responses against human papilloma virus and human papilloma virus induced laryngeal cancer. *J Laryngol Otol* 2010;124:659–662.
166. Flores-de la TC, Hernandez-Hernandez DM, Gallegos-Hernandez JF. Human papilloma virus in patients with epidermoid head and neck carcinoma: a prognostic factor? *Cir Cir* 2010;78:221–228.
167. Liu B, Lu Z, Wang P, Basang Z, Rao X. Prevalence of high-risk human papillomavirus types (HPV-16, HPV-18) and their physical status in primary laryngeal squamous cell carcinoma. *Neoplasma* 2010;57:594–600.
168. Morshed K, Polz-Dacewicz M, Szymanski M, Smolen A. Usefulness and efficiency of formalin-fixed paraffin-embedded specimens from laryngeal squamous cell carcinoma in HPV detection by IHC and PCR/DEIA. *Folia Histochem Cytobiol* 2010;48:398–402.
169. Peralta R, Baudis M, Vazquez G, et al. Increased expression of cellular retinol-binding protein 1 in laryngeal squamous cell carcinoma. *J Cancer Res Clin Oncol* 2010;136:931–938.
170. Deng Z, Hasegawa M, Matayoshi S, et al. Prevalence and clinical features of human papillomavirus in head and neck squamous cell carcinoma in Okinawa, southern Japan. *Eur Arch Otorhinolaryngol* 2011; 268:1625–1631.
171. Duray A, Descamps G, Arafa M, et al. High incidence of high-risk HPV in benign and malignant lesions of the larynx. *Int J Oncol* 2011;39:51–59.
172. Lee SY, Cho NH, Choi EC, Kim WS, Kim SH. Is human papillomavirus a causative factor of glottic cancer? *J Voice* 2011;25:770–774.
173. Lill C, Kornek G, Bachtiary B, et al. Survival of patients with HPV-positive oropharyngeal cancer after radiochemotherapy is significantly enhanced. *Wien Klin Wochenschr* 2011;123:215–221.
174. Rades D, Seibold ND, Gebhard MP, Noack F, Schild SE, Thorns C. Prognostic factors (including HPV status) for irradiation of locally advanced squamous cell carcinoma of the head and neck (SCCHN). *Strahlenther Onkol* 2011;187:626–632.
175. Ribeiro KB, Levi JE, Pawlita M, Koifman S, Matos E, Eluf-Neto J, et al. Low human papillomavirus prevalence in head and neck cancer: results from two large case-control studies in high-incidence regions. *Int J Epidemiol* 2011;40:489–502.
176. Schlecht NF, Brandwein-Gensler M, Nuovo GJ, et al. A comparison of clinically utilized human papillomavirus detection methods in head and neck cancer. *Mod Pathol* 2011;24:1295–1305.
177. Snietura M, Piglowski W, Jaworska M, et al. Impact of HPV infection on the clinical outcome of p-CAIR trial in head and neck cancer. *Eur Arch Otorhinolaryngol* 2011;268:721–726.
178. Hassumi-Fukasawa MK, Miranda-Camargo FA, Guimaraes MC, et al. Possible implication of Mdm2 as a prognostic marker in invasive laryngeal carcinoma. *Eur Arch Otorhinolaryngol* 2012;269:1795–1804.
179. Stephen JK, Chen KM, Shah V, et al. Human papillomavirus outcomes in an access-to-care laryngeal cancer cohort. *Otolaryngol Head Neck Surg* 2012;146:730–738.
180. Tezal M, Scannapieco FA, Wactawski-Wende J, et al. Local inflammation and human papillomavirus status of head and neck cancers. *Arch Otolaryngol Head Neck Surg* 2012;138:669–675.
181. Rautava J, Kuuskoski J, Syrjanen K, Grenman R, Syrjanen S. HPV genotypes and their prognostic significance in head and neck squamous cell carcinomas. *J Clin Virol* 2012;53:116–120.
182. van Monsjou HS, van Velthuysen ML, van den Brekel MW, Jordanova ES, Melief CJ, Balm AJ. Human papillomavirus status in young patients with head and neck squamous cell carcinoma. *Int J Cancer* 2012;130:1806–1812.
183. Sun J, Xiong J, Zhen Y, Chen ZL, Zhang H. P53 and PCNA is positively correlated with HPV infection in laryngeal epitheliopapillomatous lesions in patients with different ethnic backgrounds in Xinjiang. *Asian Pac J Cancer Prev* 2012;13:5439–5444.
184. Si-Mohamed A, Badoual C, Hans S, Pere H, Tartour E, Brasnu D. An unusual human papillomavirus type 82 detection in laryngeal squamous cell carcinoma: case report and review of literature. *J Clin Virol* 2012; 54:190–193.
185. Shi Q, Xiao K, Wei W, et al. Associations of TP53 mutations, codon 72 polymorphism and human papillomavirus in head and neck squamous cell carcinoma patients. *Oncol Rep* 2013;30:2811–2819.
186. Ankola AA, Smith RV, Burk RD, Prystowsky MB, Sarta C, Schlecht NF. Comorbidity, human papillomavirus infection and head and neck cancer survival in an ethnically diverse population. *Oral Oncol* 2013;49:911–917.
187. Quintero K, Giraldo GA, Uribe ML, et al. Human papillomavirus types in cases of squamous cell carcinoma of head and neck in Colombia. *Braz J Otorhinolaryngol* 2013;79:375–381.
188. Mehrad M, Carpenter DH, Chernock RD, et al. Papillary squamous cell carcinoma of the head and neck: clinicopathologic and molecular features with special reference to human papillomavirus. *Am J Surg Pathol* 2013;37:1349–1356.
189. Watson RF, Chernock RD, Wang X, et al. Spindle cell carcinomas of the head and neck rarely harbor transcriptionally-active human papillomavirus. *Head Neck Pathol* 2013;7:250–257.
190. Anantharaman D, Gheit T, Waterboer T, et al. Human papillomavirus infections and upper aero-digestive tract cancers: the ARCAGE study. *J Natl Cancer Inst* 2013;105:536–545.
191. Huebbers CU, Preuss SF, Kolligs J,et al. Integration of HPV6 and downregulation of AKR1C3 expression mark malignant transformation in a patient with juvenile-onset laryngeal papillomatosis. *PLoS One* 2013;8: e57207.
192. Upile NS, Shaw RJ, Jones TM, et al. Squamous cell carcinoma of the head and neck outside the oropharynx is rarely human papillomavirus related. *Laryngoscope* 2014;124:2739–2744.
193. Salazar CR, Anayannis N, Smith RV, et al. Combined P16 and human papillomavirus testing predicts head and neck cancer survival. *Int J Cancer* 2014;135:2404–2412.
194. Shaughnessy JN, Farghaly H, Wilson L, et al. HPV: a factor in organ preservation for locally advanced larynx and hypopharynx cancer? *Am J Otolaryngol* 2014;35:19–24.
195. Mohamadian Roshan N, Jafarian A, Ayatollahi H, Ghazvini K, Tabatabaee SA. Correlation of laryngeal squamous cell carcinoma and infections with either HHV-8 or HPV-16/18. *Pathol Res Pract* 2014;210: 205–209.
196. Gheit T, Abedi-Ardekani B, Carreira C, Missad CG, Tommasino M, Torrente MC. Comprehensive analysis of HPV expression in laryngeal squamous cell carcinoma. *J Med Virol* 2014;86:642–646.
197. Laskaris S, Sengas I, Maragoudakis P, et al. Prevalence of human papillomavirus infection in Greek patients with squamous cell carcinoma of the larynx. *Anticancer Res* 2014;34:5749–5753.
198. Chung CH, Zhang Q, Kong CS, et al. p16 protein expression and human papillomavirus status as prognostic biomarkers of nonoropharyngeal head and neck squamous cell carcinoma. *J Clin Oncol* 2014;32:3930–3938.
199. Lassen P, Primdahl H, Johansen J, et al.; Danish Head and Neck Cancer Group (DAHANCA). Impact of HPV-associated p16-expression on radiotherapy outcome in advanced oropharynx and non-oropharynx cancer. *Radiother Oncol* 2014;113:310–316.
200. Hernandez BY, Goodman MT, Lynch CF, et al.; HPV Typing of Cancer Workgroup. Human papillomavirus prevalence in invasive laryngeal cancer in the United States. *PLoS One* 2014;9:e115931.
201. Deng Z, Hasegawa M, Aoki K, et al. A comprehensive evaluation of human papillomavirus positive status and p16INK4a overexpression as a prognostic biomarker in head and neck squamous cell carcinoma. *Int J Oncol* 2014;45:67–76.
202. Rodrigo JP, Hermsen MA, Fresno MF,et al.. Prevalence of human papillomavirus in laryngeal and hypopharyngeal squamous cell carcinomas in northern Spain. *Cancer Epidemiol* 2015;39:37–41.
203. Young RJ, Urban D, Angel C, et al. Frequency and prognostic significance of p16(INK4A) protein overexpression and transcriptionally active human papillomavirus infection in laryngeal squamous cell carcinoma. *Br J Cancer* 2015;112:1098–1104.
204. Kanyilmaz G, Ekinci O, Muge A, Celik S, Ozturk F. HPV-associated p16 INK4A expression and response to therapy and survival in selected head and neck cancers. *Asian Pac J Cancer Prev* 2015;16:253–258.
205. Huedo-Medina T, Sanchez-Meca J, Marin-Martinez F, Botella J. Assessing heterogeneity in meta-analysis: Q statistic or I2 index? *CHIP Documents*. Paper 19. DigitalCommons@UConn website. Available at: http://digitalcommons.uconn.edu/chip_docs/19. Published June 1, 2006. Accessed March 30, 2015.
206. Field AP. Meta-analysis of correlation coefficients: a Monte Carlo comparison of fixed- and random-effects methods. *Psychol Meth* 2001;6:161–180.
207. Field AP. The problems in using fixed-effects models of meta-analysis on real-world data. *Understand Stat* 2003;2:77–96.
208. Cochran WG. The combination of estimates from different experiments. *Biometrics* 1954;10:101–129.
209. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *Brit Med J* 2003;327:557–560.
210. Smith EM, Rubenstein LM, Ritchie JM, et al. Does pre-treatment seropositivity to human papilloma virus have prognostic significance for head and neck cancers? *Cancer Epidemiol Biomarkers Prev* 2008;17:2087–2096.
211. Koskinen WJ, Chen RW, Leivo I, et al. Prevalence and physical status of human papillomavirus in squamous cell carcinomas of the head and neck. *Int J Cancer* 2003;107:401–406.